# SPECIFICATION

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# [COMMERCIAL PROCESS FOR ISOLATION AND PURIFICATION OF GLABRIDIN WITH HIGH TYROSINASE INHIBITORY ACTIVITY AND ITS COSMETIC COMPOSITION AND METHODS OF USE]

## Background of Invention

### [Field of Invention]

[0001] The present invention relates to cosmetic, particularly skin care, treatment and compositions. More particularly, the current invention discloses the commercial non-chromatographic isolation of Glabridin and its use as tyrosinase inhibiting component in cosmetic formulations. Tyrosinase inhibitors have been highly useful in providing fairness to the skin, ameliorating skin pigmentation disorders such as age spots, freckles, dark circles around the eyes or armpits, or in any part of the body darkened due to exposure to UV rays or through increased melanin deposition.

### [Description of Prior Art]

[0002] Isoflavones are a larger and distinctive subclass of flavonoids. These compounds possess a 3-phenyl chromane skeleton that is biogenetically derived by rearrangement of the flavonoid 2-phenyl chromane system (1,2 diaryl rearrangement). Isoflavonoids are almost entirely distributed to the subfamily Papilionaceae of Leguminoseae family. Several flavonoids are potent inhibitors of lipoxygenase or cyclooxygenase, or both. These properties explain their anti-inflammatory and

antiallergenic activity. Glabridin an isoflavan found in licorice extracts is reported to have anti-inflammatory, antioxidant and tyrosinase inhibitory properties (Yokota, T. et al. Pigment Cell Res. 11(6):355,361, 1998; Vaya, J. Free Rad. Biol. Med. 23(2):302-313, 1997). Methods to isolate isoflavans and other tyrosinase inhibitors have been described in literature (Mitscher, L. et al. J. Nat. Prod. 43(2):259-269, 1980; Shirota, S. et al. Biol. Pharm. Bull. 17(2):266-269, 1994; Saitoh, T. et al. Chem. Pharm. Bull. 24 (4):742-755). Melanin is one of the key determinants of skin color. L-dopa and dopachrome are produced as oxidation intermediates in the enzymatic conversion of L-tyrosine to melanin by the enzyme, tyrosinase. The oxidation-reduction potential of skin whitening agents is related to their inhibitory action on melanin biosynthesis, through tyrosinase inhibition (Sakuma Y. et al. Arch. Pharm. Res. 22(4):335-339) .

[0003] US 4428876:describes the process for isolating flavonoids by extraction with an aqueous alkali solution and then combining with absorption-elution steps using non-polar or slightly polar adsorbent resins.

[0004] Japanese patent application 63-140404 describes the isolation of glabridin and glabrene from licorice roots and the use of these compounds in cosmetics and drugs for external use.

[0005] Japanese patent application 04-332197 describes a composition containing glabridin and an antiphlogistic which is capable of suppressing inflammation and melanogenesis and melanin pigmentation caused by external irritation of ultraviolet rays, etc., by synergic action of these essential ingredients.

[0006] JP 6145038A2 describes the process of extracting Glabridin by solvent extraction followed by normal and reverse silica gel chromatography and crystallization. Skin whitening compositions containing glabridin at levels of 0.001 to 3.0%, are described.

[0007] US patent # 5,609,875 describes skin whitening compositions containing Glycyrrhiza extracts that also prevent the formation of skin spots.

[0008] WO02074277 describes glabridin-based depigmenting dermatological composition, comprising at least 0.04 wt % glabridin.

[0009] US patent #5,093,109 provides anti-aging composition comprising water, anti-

aging agent, a sunscreen, a preservative, a thickener, an antioxidant and an emulsifier.

[0010] US patent application # 2002054891 describes the use of antioxidant composition in the form of an oil in water emulsion containing hesperitin, tetrahydrocurcumin, tetrahydrodemethoxycurcumin, tetrahydrobisdemethoxycurcumin or mixtures thereof for protection of keratinous tissue against environmental factors such as smoke, smog and UV radiation.

[0011] US patent # 4742066 discloses a method for inhibiting the generation of free radicals in the skin. The method comprises applying a composition that may be in the form of an emulsion in which the free radical inhibitor may be ethoxyquin, or "Trolox C".

[0012] US patent # 4424234 discloses cosmetic formulations containing hydroxy alkanoic acid and may contain tocopherol, propyl gallate, ascorbyl palmitate, butylated hydroxytoluene or butylated hydroxyanisole as antioxidants and could be useful for treatment of dry skin.

[0013] US patent # 5861415 disclosed the pharmaceutical use as a bioprotectant of a mixture of curcumin, demethoxy curcumin and bisdemethoxycurcumin derived from the rhizomes of turmeric.

[0014] All of the prior art describe the use of a crude extract containing up to 10% of glabridin by weight of the extract. None of the prior art describe the use of highly pure glabridin, isolated from licorice roots, with very high tyrosinase inhibitory activity. In the present invention, we describe a novel method for isolating glabridin of high purity, up to 90%, possessing high tyrosinase activity, and surprisingly, low antioxidant activity.

## Summary of Invention

[0015] The present invention discloses the commercial isolation of glabridin of higher purity and its use in compositions as an inhibitor of melanin synthesis by inhibiting the rate limiting enzyme tyrosinase. The recovery of glabridin by a non-chromatographic method and a non-chromatographic, non-invasive method using

supercritical fluid extraction are described. Compositions containing glabridin and their use in skin care are described.

[0016] The object of the present invention is to provide a commercially viable process for isolation of Glabridin and provide cosmetic compositions having tyrosinase inhibitors.

[0017] It is another object of the present invention to provide compositions that have number of benefits in connection with skin care and prevent the damage to the keratinous tissue.

[0018] The compositions of this invention may be in the form of gel, lotion, anhydrous sticks, oil based sprays, oil-in-water or water-in-oil emulsion.

[0019] These and other objects of the present invention are achieved by a cosmetic composition comprising essentially tyrosinase inhibitors selected from the group comprising of:

[t8]

| | |
|---|---|
| I. | Glabridin 4% or 20% or 40% or 90% |
| II. | Tetrahydrocurcumin |
| III. | Tetrahydrodemethoxy curcumin |
| IV. | Tetrahydrobisdemethoxy curcumin |
| V. | Ellagic acid |
| VI. | Soya Isoflavones |
| VII. | Demethoxy curcumin |
| VIII. | Bisdemethoxy curcumin |
| IX. | Mixtures of I with one or more ingredients selected from the listed ingredients II-VIII. |

[0020] The composition may also include antioxidants, sunscreens, emulsifiers, preservatives, anti-wrinkle agents, thickeners and fragrances.

## Brief Description of Drawings

[0021] Figure 1: Chemical structures of some natural tyrosinase inhibitors

[0022] Figure 2: HPLC chromatograms for 4% glabridin.

[0023] Figure 3 : HPLC chromatogram for 20% glabridin

[0024] Figure 4 : HPLC chromatogram for 40% glabridin

[0025] Figure 5: HPLC chromatogram for 80% glabridin

## Detailed Description

[0026] The present invention discloses novel non-chromatographic, commercially viable processes to isolate Glabridin of different purities. In addition, the present invention includes, in part, a skin care composition containing from about 0.01% to about 10% preferably from 0.1 to 3% most preferably from 0.1 to 0.5% by weight of a composition of purified glabridin containing 4, 20,40 or 90% glabridin by weight and a cosmetically acceptable vehicle. The purity of glabridin in licorice extracts used in the compositions of the present invention, is selected at optimal levels to support the multiple functions of tyrosinase inhibition, antioxidant effects and UV protective effects.

[0027] Glabridin of varying strengths have been studied for their skin whitening properties by examination for tyrosinase inhibitory activity and evaluating the critical wave length in UVA / UVB ratio to ascertain their UV protectant action.

[0028] Further, in accordance with the present invention, there has been disclosed, cosmetic compositions, preferable in the form of oil-in-water emulsion. The composition contains glabridin with or without one or more of the following tyrosinase inhibitors: Tetrahydrocurcumin, Tetrahydrodemethoxycurcumin, Tetrahydrobisdemethoxycurcumin, curcumin, demethoxycurcumin, bisdemethoxycurcumin, ellagic acid, soy isoflavones. The composition may also include one or more triterpenic acids as anti-wrinkle component, antioxidants, sunscreens, emulsifiers, preservatives and thickeners.

[0029] The compositions of the present invention offer a number of benefits in providing skin fairness and preventing uneven pigmentation due photoaging; additionally, providing antioxidant, UV protectant and long term protection against UVA induced loss of elasticity, wrinkle and line development.

[0030] Glabridin, in prior art was obtained from Licorice roots by extraction and chromatographic separation of impurities. However, chromatographic methods for purification are time consuming and cumbersome.

[0031] The present invention uses solvent solvent extraction especially from acidic medium to obtain various grades of Licorice extract containing 4%, 20%, 40% and 90% Glabridin. Interestingly, it was found that though the use of higher percentage of

Glabridin containing extracts show increasing activity as tyrosinase inhibitor, they show simultaneously lower antioxidant property as well as decrease in UVA protection range as reflected by the critical wavelength measurement (Table 1). Glabridin isolated is used in its pure form in the present composition preferably as a tyrosinase inhibitor, but most preferably as a 20% - 40% Glabridin containing Licorice extract to afford additional UV protection and antioxidant properties in concentrations of 0.1 to 5% preferably in ranges of 0.1 to 2% w/w of the composition.

[0032] Tetrahydrocurcumin (THC), tetrahydrodemethoxycurcumin (THDMC), Tetrahydrobisdemethoxycurcumin (THBDMC) as represented in Figure 1, are obtained by hydrogenation of curcuminoids obtained from Curcuma longa. The tyrosinase inhibitor is a mixture of these three compounds typically in a ratio of 75% - 95% w/w of THC, about 0-20% wt of THDMC and about 0-10% of THBDMC, most preferably a combination of Glabridin and THC or Glabridin with THC, THDMC & THBDMC. The preferred concentrations of THC in the composition is 0.01 - 1% w/w, THDMC in the composition is 0.1- 2% w/w, of THBDMC in the composition is 0.1- 2% w/w.

[0033] Ellagic acid whose structure is shown in Figure 1, obtained as pure material from Terminalia chebula polyphenols fraction, is used as a tyrosinase inhibitor, more preferably with Glabridin and THC, at 0.1- 0.5% w/w in the composition.

[0034] Soy Isoflavones from soybeans which may be represented by the structures shown in Figure 1, consisting of 20 to 40% aglycones such as Genistein and Daidzein, may be used as a tyrosinase inhibitor in combination with Glabridin or THC or both, and can be used in 0.5 to 2% w/w in the composition.

[0035] The emulsifiers may be anionic, cationic, zwitterionic, amphoteric or non ionic, where the composition is prepared in the form of an o/w or w/o emulsion. Suitable emulsifiers include polymeric acrylate emulsifiers, polysorbate 20, sorbitan tristearate, polyethylene glycol 40 stearate sorbitan trioleate, glyceryl monostearate, isopropyl myristate, Tween 80, Lecithin, polythylene glycol 20 stearyl ether (Brij 78, Steareth 20). Polyethylene glycol ether of lauryl alcohol (Laureth 23) or others that are approved for cosmetic use. The total amount of emulsifier will vary from about 1% to 10% w/w preferably about 2.5 to 3.0% w/w.

[0036] The preservatives are any preservative suitable for use in a topically applied cosmetic product. Such preservatives include Imidurea (ICI Ltd), ethanol, benzyl alcohol, disodium EDTA, methyl paraben, ethyl paraben, or butyl paraben. The preservative will be present in amounts effective to prevent bacterial growth in ranges from 1% to 3% w/w.

[0037] The composition could have a thickener to obtain the optimum viscosity and shearing properties when applied to the skin. Preferably Carbopol 934, Carbopol 940, Carbopol 950, Carbopol 980, Carbopol 951, Stearyl alcohol, stearic acid, hydroxy ethyl cellulose, Xyloglucans, β -glycans, propylene glycol monostearate, Carboxymethylcellulose, myristyl stearate and cetyl stearate in amounts ranging from 0.3% - 3% w/w.

[0038] Besides, thickener, preservatives and emulsifiers, the composition may include water from a range of 25% - 90% w/w or oils like coconut oil fraction (rich in lauric acid-Lauriforte ® ), Petroselinic acid triglycerides from coriander oil, Punicic acids and triglycerides from pomegranate oil, Ximenynic acid from sandal seeds, galanga oil from Kaempferia galanga rhizomes depending on the type of emulsion.

[0039] The present composition will preferably contain one or more sunscreens for protection against both UVA and UVB rays which includes but not limited to octyl salicylate, 6-alkyl salicylates from Ginkgo or Ancardium occidentale, ethylhexyl methoxy cinnamate, ethyl-p-methoxycinnamates, cinnamoyl cinnamates, oxybenzone, avobenzone and other diferuloyl methane derivatives or other sunscreens or sunscreen boosters in the preferred concentration in w/w : Oxybenzone 2-10% , sulisobenzone 5-10%, dioxybenzone 1-3% , octyl methoxycinnamate 2-10%, ethyl-p-methoxycinnamate or galanga extract 0.5-7.5% , isoamyl p-methoxycinnamate 5-7.5%, octyl salicylate 3-5%, 6-alkyl salicylates like anacardic acids and ginkgolic acid esters 2-7.5%, homomenthyl salicylate 4-15%, Triazine 1-10% among others.

[0040] Additionally, the composition may contain anti-wrinkle agents such as ursolic acid, rosemary, sage extract, melissa extract, oleanolic acid, olive extract, maslinic acid, lupeol, silver birch extract, betulin, betulinic acid, ursolic acid methyl esters and salts, arjunolic acids, boswellic acids, lotus seed extract among others at the level of

0.5 to 5% w/w of the composition, based on intended use.

[0041] The composition may contain additional antioxidants like tocotrienols, tocopherols, gamma oryzanol, ascorbyl monopalmitate, tomato extract, lycopene, lutein, zeaxanthin, carotenoids, garcinol and related polyisoprenylated benzophenones, dibenzo- α -pyrones, xanthones like mangiferin, ellagitannins like emblicannins from amla, chebulic acid from Terminalia chebula, rosmarnic acid, ferulic acid, carnosic acid, alpha lipoic acid amongst others in concentrations of 0.1 to 2% w/w.

[0042] The composition of the present invention may also contain humectants including but not limited to glycerol, propylene glycol, butylene glycol, 1,2-hexanediol, sorbitol, dibutyl phthalates, collagen amongst others in concentrations of 3-7% w/w.

[0043] Emollients in the composition would be between 3% and 12% w/w. Suitable emollients include mineral oil, coriander oil, pomegranate seed oil, momordica seed oil, flax seed oil, sandal seed oil, sesame oil, kokum butter, squalane, avocado oil, isopropyl myristate, caprilic / caproic esters, dimethicone among others.

[0044]

*Example 1: Isolation and purification of glabridin from licorice roots by solvent extraction and crystallization* Powdered roots of Glycyrrhiza glabra are extracted with organic solvents like hexane, methanol, isopropyl alcohol, ethanol, acetone, toluene, ethyl acetate preferably with acetone. The solvent is distilled out and the resultant extract containing 4% Glabridin, is dissolved in 5-50% v/v aqueous acid solutions like citric acid, tartaric acid, hydrochloric acid, acetic acid preferably with 5-20% vinegar. Addition of water to this acidic layer yields a precipitate of 20% Glabridin. For obtaining higher purity Glabridin, the acidic layer is extracted with a mixture of non-polar and polar solvents selected from hexane, toluene, ethyl acetate, n-butanol preferably mixtures of hexane and toluene in ratios of 10:90 to 90:10. Mobile Phase : A 100% water filter and sonicate to degas. Mobile Phase : B 100% acetonitrile filter and sonicate to degas. Total flow rate is 1.2mL per minute

[t13]

| Time | Conc. |
|---|---|
| 0.01 minute | 50.0% |
| 15.0 minute | 50.0% |
| 30.0 minute | 80.0% |
| 40.0 minute | 80.0% |
| 45.0 minute | 50.0% |
| 50.0 minute | Stop |

[0045] *Standard Preparation for Glabridin :* Transfer about 20mg of Glabridin standard, accurately weighed, to 100mL volumetric flask add 60mL of methanol to dissolve and dilute to volume with methanol.

[0046] *Sample Preparation :* Transfer quantity equivalent to 20mg of pure standard of Glabridin accurately weighed, to 100mL volumetric flask add 60mL of methanol and sonicate to dissolve, dilute to volume with methanol.

[0047] *Chromatographic System* :The liquid chromatograph is equipped with 230nm UV detector and a 4.0mm x 250mm C18 (Merck/Supelco) column. Total flow rate is set to 1.2mL/min. Chromatograph the Standard preparation, and record the peak responses, as directed under the procedure, the relative standard deviation for replicate injection is not more than 1.0% *Procedure* : Separately inject equal volumes (20 µ ) of the standard preparations and the sample preparation into the Chromatograph, record the chromatograms, and measure the responses for the peak corresponding to Glabridin.

[0048] Calculate the percentage of Glabridin as follows

$$\frac{\text{Area of the peak corresponding to Glabridin in sample X Standard Glabridin Conc (mg/ml) X Stl. Assay(Glabridin)}}{\text{Area of the peak corresponding to Glabridin in standard X Sample Concentration (mg/ml)}}$$

Approximate retention time for Glabridin : 13 to 15 minutes. The HPLC chromatograms for glabridin of various purities are shown in Figures 2-5.

[0049] *Example 2: Isolation and purification of glabridin from licorice roots by Supercritical $CO_2$ extraction followed by solvent solvent extraction and crystallization.* Supercritical fluid extraction using carbon dioxide with and without modifiers such as ethanol, acetone or ethyl acetate extract the Glabridin from the roots of Licorice are described. The extractions were done at temperatures ranging from 25 to 120 ° C, preferably between 45- 55 ° C, the extraction fluid pressure

between 100 to 300 bar preferably at 300 bars with or without co-solvents preferably 5% ethanol, for 1-5 hrs, preferably for 3 hrs and at carbon dioxide flow rate of 1-4kg/h, preferably at 2 kg/hr. The total content of Glabridin in the extract was analyzed by HPLC. The extracts were further purified by solvent-solvent extractions and crystallization as described earlier.

[0050] *Example 3 Concentration dependent functional properties of glabridin*

*Measurement of tyrosinase Activity:* The tyrosinase activity was measured according to the published method. Mushroom tyrosinase (EC 1.14.18.1) were purchased from Sigma Aldrich, USA. The enzyme was diluted with 67 mM phosphate buffer (pH 6.8) to 500U/ml and used. L-Tyrosine (Thomas Becker, Mumbai, India) 0.55mM solution and DMSO AR grade (SD Fine Chemicals, Mumbai, India) was used in this study. Kojic acid and Vitamin C (Sigma Aldrich, USA) were used for comparison. Glabridin 4%, 20%, 40% and 90% were used in the study. Tetrahydrocurcuminoids, Tetrahydrocurcumin, ethyl p-methoxycinnamate, ellagic acid, soy isoflavones were obtained by isolation according to the published procedures.

[0051] Tyrosinase inhibiting assay was carried out as follows. Briefly, the incubation mixture of 3 ml containing 0.55 mM L-Tyrosine, 500U/ml Tyrosinase, 67mM phosphate buffer (pH6.8) and inhibitors were incubated at 37 ° C for 20 min. and absorbance was recorded at 475 mm (S). Inhibitors were prepared in DMSO in concentrations of $10^{-2}$ to $10^{-10}$ M. Blank readings (B) were recorded in the absence of inhibitors but contained equivalent volume of DMSO. To neutralize the inherent color of the inhibitors, absorbance of a mixture containing inhibitor and buffer and without tyrosinase were recorded (C).

[0052] The results were calculated from the formula : % inhibition = B-(S-C)/B X100. The results are expressed as inhibitory concentration ($IC_{50}$)

[0053] *Measurement of antioxidant activity:* Antioxidant activity was measured by the DPPH radical scavenging activity. 1,1-Diphenyl 2-picrylhydrazyl (DPPH) purchased from Sigma Aldrich USA was used in study. Briefly, to test the radical scavenging activity, the samples prepared in ethanol, is added to 0.1 mM DPPH in ethanolic solution. The mixture was incubated at 37 ° C for 30 min. and the free radical concentration measured by spectrophotometer at 516nm. Free radical scavenging

activity ($SC_{50}$) was defined as the concentration needed for 50% of free radicals to be inhibited / scavenged. Low concentration (mcg) required for SC50 indicate high antioxidant activity, and high concentration (mcg) required for SC50 suggest low antioxidant activity. Measurement of critical wavelength and UVA/UVB ratio (sunscreen potential):The critical wavelength λ c is a measure of sunscreen"s extinction capacity in UVA range in relation to its overall extinction between 290nm and 400nm. The extinction capacity is given by the area under the extinction curve. λ c is calculated as the wavelength at which this area corresponds to 90% of the total area $A_{290-400}$. The higher the critical wavelength of a sunscreen, the better its UVA performance.

[0054] The critical wavelength is calculated as follows:

$$\int_{290}^{\lambda c} E(\lambda)d\lambda = 0{,}9 \bullet \int_{290}^{400} E(\lambda)\,d\lambda$$

The UVA/UVB ratio defines the performance of a sunscreen in the UVA range (320–400nm) in relation to its performance in the UVB range (290–320nm). It is calculated as the ratio between the area defined by the UVA and UVB extinction capacity. They are calculated as follows:

$$\text{UVA/UVB ratio} = \frac{\int_{320}^{400} A(\lambda)d\lambda \Big/ \int_{320}^{400} d\lambda}{\int_{290}^{320} A(\lambda)d\lambda \Big/ \int_{290}^{320} d\lambda}$$

*Table 1: Comparative studies of glabridin of varying purity for tyrosinase activity, antioxidant activity and UV protectant properties*

[t5]

| | Glabridin 4 % | Glabridin 20 % | Glabridin 40 % | Glabridin 80 % |
|---|---|---|---|---|
| $IC_{50}$ (mcg) | 1 | 0.15 | 0.045 | 0.035 |
| $SC_{50}$ (mcg) | 29 | 35 | 49 | 100 |
| UVA / UVB | 0.518 | 0.326 | 0.275 | 0.160 |
| Critical wavelength | 374 | 360 | 357 | 325 |

[0055] The use of higher purity of Glabridin in licorice extract, increases the tyrosinase inhibition as reflected by decrease in the IC50 values. On the other hand, use of lower percentage of glabridin improves the antioxidant activity (SC50), critical wavelength indicating a broad spectrum protection against UV rays.

[0056] *Example 4 - Skin whitening composition with enhanced dermal penetration and antioxidant activity*

[t16]

| | |
|---|---|
| Glabridin 40 % | 1.0% |
| Tetrahydrocurcumin | 0.5% |
| Tetrahydropiperine | 0.1% |
| Lauric acid ester fraction from Coconut oil (Lauriforte®) | Q.S 100% |

The skin fairness oil blend inhibited tyrosinase activity at low concentration $_{50}$ = 10 mcg. The antioxidant (Free radical scavenging activity, SC $_{50}$ ) is 1.42 mg. The unique feature of this oil is that it is a non-sticky, non-greasy, chemically stable oil, useful as a massage oil. Tetrahydropiperine is obtained by hydrogenation of piperine and is known to increase the permeation of actives across the epidermis.

[0057] *Example 5 - Skin care composition containing fairness components with broad-spectrum UV protection and antioxidant activity.*

[t9]

| Composition | Percentage |
|---|---|
| Glabridin 40 % | 5 % |
| Tetrahydrocurcumin | 4.5 % |
| Tetrahydro demethoxycurcumin | 0.35% |
| Tetrahydrobis demethoxycurcumin | 0.15% |
| Ellagic acid | 1 % |
| Galanga ester | 2 % |
| Boswellic acids | 2 % |
| N methyl pyrollidone | Q.S to 100% |

[0058] The blend had tyrosinase inhibition expressed as IC $_{50}$ =1.8 mcg.

[0059] *Example 6 Skin care composition containing anti-aging, anti-oxidants, anti-wrinkle and skin rejuvenating components.*

[t10]

| | |
|---|---|
| Glabridin 40% | 0.5 % |
| Tetrahydrocurcuminoids | 0.1 % |
| Amla Extract | 1 % |
| Ursolic acid | 1.5 % |
| Sesamin complex containing sesamine and sesamoline | 0.5 % |
| Cetyl alcohol as excepient | QS to 100% |

[0060] The tyrosinase inhibition of the blend expressed as IC $_{50}$ =15mcg

[0061] *Example 7: Skin care composition containing natural tyrosinase inhibitors blend*

[t11]

| | |
|---|---|
| Tetrahydrocurcumin | 0.001% |
| Glabridin 40% | 0.1% |
| Cetyl alcohol | Q.S.to 100% |

[0062] Tyrosinase activity of the natural tyrosinase inhibitor blend expressed as IC $_{50}$

=3.0mcg

[0063] *Example 8: Skin care composition containing natural tyrosinase inhibitor blend*

[t17]

| | |
|---|---|
| Tetrahydrocurcuminoids | 0.01% |
| Ellagic acid | 0.5% |
| Glabridin 40% | 0.1% |
| Cetyl alcohol | Q.S |

[0064] Tyrosinase activity of the natural tyrosinase inhibitor blend expressed as $IC_{50}$ 13.6 mcg

[0065] *Example 9: Skin care composition containing antioxidant, skin fairness and anti-wrinkle blend*

[t12]

| | |
|---|---|
| Green tea extract | 0.5% |
| Glabridin 40% | 0.05% |
| Ursolic acid (90%) | 2.0% |
| Acetyl keto-β-Boswellic acid | 0.5% |

[0066] Tyrosinase activity of the blend expressed as $IC_{50}$ =50 mcg

[0067] *Example 10: Skin care composition containing natural moisturizer and skin fairness enhancer*

[t21]

| | |
|---|---|
| Glabridin 40% | 0.5% |
| Xylosin® | 2.0% |
| (Xyloglucan from tamarind seed, a natural moisturizer) | |
| Cetyl alcohol | QS |

[0068] Tyrosinase activity of the blend expressed as $IC_{50}$ = 13 mcg.

[0069] *Example 11: Skin care composition containing anti-oxidants, anti-wrinkle, anti-aging and fairness blend.*

[t19]

| | |
|---|---|
| Sesamin / Sesamolin complex | 0.5% |
| Amla extract (containing emblicannins) | 1% |
| Glabridin 40% | 0.5% |
| Tetrahydrocurcumin | 0.01% |
| Ursolic acid | 1.5% |
| Cetyl alcohol | QS |

[0070] Tyrosinase inhibition of the blend expressed as $IC_{50}$ = 17.9 mcg

[0071] *Example 12: Skin Care composition containing phytoestrogens, anti-wrinkle, skin fairness blend.*

[t15]

| | |
|---|---|
| Soy isoflavones 40% | 0.5% |
| Tetrahydrocurcumin | 0.07% |
| Tetrahydro demethoxy curcumin | 0.02% |
| Tetrahydro bisdemethoxy curcumin | 0.005% |
| Glabridin 4% | 0.5% |
| Betulin | 1% |
| Cetyl alcohol | QS |

[0072] Tyrosinase inhibition of the blend expressed as $IC_{50}$ = 18 mcg.

[0073] *Example 13: Skin care composition containing skin fairness component, hair growth regulator, wrinkle control blends.*

[t18]

| | |
|---|---|
| Glabridin 40% | 0.5% |
| Oleanolic acid 80% | 0.5% |
| Betulin | 0.8% |
| Betulinic acid | 0.15% |
| Lupeol | 0.05% |
| Cetyl alcohol | QS |

[0074] Tyrosinase inhibition of the blend expressed as $IC_{50}$ = 18.8 mcg

[0075] *Example 14: Skin care composition containing tyrosinase inhibitor, ornithine decarboxylase inhibition and anti-inflammatory blends.*

[t20]

| | |
|---|---|
| Glabridin (40%) | 0.5% |
| Oleanolic acid (80%) | 1.0% |
| Boswellic acids | 0.5% |
| Cetyl alcohol | QS |

Tyrosinase inhibition of the blend expressed as $IC_{50}$ = 23.1 mcg

[0076] *Example 15: Skin care composition containing tyrosinase inhibitor, anti-aging complex and anti-wrinkle blend.*

[t1]

| | |
|---|---|
| Glabridin 20% | 0.01% |
| Coriander oil (petroselenic acid ester) | 0.5% |
| Tetrahydrocurcuminoids | 0.1% |
| Ursolic acid | 0.5% |
| Cetyl alcohol | QS |

[0077] Tyrosinase inhibition of the blend expressed as $IC_{50}$ =86.1 mcg

[0078] *Example 16: Skin care composition containing tyrosinase inhibitor and sunscreen boosters.*

[t2]

| | |
|---|---|
| Glabridin 4% | 0.5% |
| Galanga ester (ethyl p-methoxy cinnamate) | 5.0% |
| Cetyl alcohol | QS to 100% |

[0079] Tyrosinase inhibition of the blend expressed as $IC_{50}$ = 16.1 mcg.

[0080] *Use of the invention in formulations* :

[0081]

*Example 17: Fairness cream*

[t3]

| Ingredients | %w/w |
|---|---|
| Part A | |
| Mineral Oil | 2.5 |
| Lanolin Alcohol NF | 4.3 |
| Aluminum stearate | 0.1 |
| Microcrystalline wax | 4.0 |
| Ozokerite (MP 170deg. C) | 2.5 |
| Mineral oil 70ssu | 16.4 |
| Tetrahydrocurcumin | 0.01 |
| Glabridin 40% | 0.5 |
| BHT and BHA | 1.0 |
| Part B | |
| Glycerin | 1.5 |
| Magnesium sulphate | 0.7 |
| Ellagic acid | 0.5 |
| Deionized Water | 65.8 |

[0082] Procedure: Combine ingredients of Part A with mixing and heat to 70 degree centigrade. Combine ingredients of Part B with mixing and heat to 70-75 degree centigrade. Add Part B to part A with mixing and cool to 40 degree centigrade in homogenizer further to desired fill temperature.

[0083] *Example 18: Moisturizing and Fairness clear gel*

[t4]

| | |
|---|---|
| Part A | |
| Polysaccharides from tamarind (Xyloglucans) | 2.0% |
| Tetrahydrocurcumin | 0.01% |
| Glabridin 40% | 0.1% |
| Germaben II | 0.5% |
| | |
| Part B | |
| Deionized water | QS to 100% |

[0084] Heat Part B to 60 degree centigrade, and add part A to Part B under stirring and cool to the desired fill temperature.

[0085]

*Example 19: Skin Rejuvenating Lotion*

[t6]

| Ingredients | % (w/w) |
|---|---|
| Phase A | |
| Polyglyceryl-3 methyl glucose distearate | 3.5 |
| Glyceryl monostearate | 2.5 |
| Dicapryl ether | 5.0 |
| Caprylic/caproic esters | 5.0 |
| Propylene glycol Dicaprylate/dicaprate | 3.5 |
| Coriander oil | 1.0 |
| Cetyl alcohol | 1.5 |
| Tetrahydrocurcumin | 0.1 |
| Glabridin 20% | 0.5 |
| Phase B | |
| Glycerin | 3.0 |
| Propylene glycol | 3.0 |
| Blepharis edulis extract | 1.0 |
| Rosemarinic acid | 0.5 |
| Ellagic acid | 0.5 |
| Methylparaben | 0.15 |
| Deionized water | Q.S |

[0086] Combine the ingredients of A, stir and heat to 65 degree centigrade. Combine the ingredients of B, stir and heat to 65 degree centigrade. Add A to B under stirring. Homogenize avoiding foaming, cool to fill temperature.

[0087] *Example 20: Fairness Sunscreen Lotion*

[t7]

| Ingredients | % (w/w) |
|---|---|
| Phase A | |
| Polyglyceryl-3 methyl glucose distearate | 3.5 |
| Glyceryl monostearate | 2.5 |
| Dicapryl ether | 5.0 |
| Caprylic/caproic esters | 5.0 |
| Propylene glycol Dicaprylate/dicaprate | 3.5 |
| Octyl methoxycinnamate | 6.0 |
| Mangiferin | 2.5 |
| Cetyl alcohol | 1.5 |
| Tetrahydrocurcumin | 0.35 |
| Tetrahydro demethoxycurcumin | 0.1 |
| Tetrahydro bisdemethoxycurcumin | 0.05 |
| Glabridin 4% | 0.5 |
| Galanga extract | 2.0 |
| Phase B | |
| Glycerin | 3.0 |
| Propylene glycol | 3.0 |
| Methylparaben | 0.15 |
| Deionized water | Q.S |

[0088] Procedure :Combine the ingredients of A, stir and heat to 65 degree centigrade. Combine the ingredients of B, stir and heat to 65 degree centigrade. Add A to B under stirring. Homogenize avoiding foaming, cool to fill temperature.

[0089] While the invention has been described with particular reference to certain

embodiments thereof, it should be understood that changes and modifications may be made which are within the skill of the art without affecting the spirit of invention as mentioned under the claims.